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Research Paper

Myrtenal inhibits acetylcholinesterase, a known Alzheimer target

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Abstract

Objectives Inhibition of acetylcholinesterase (AChE) is a common treatment for early stages of the most general form of dementia, Alzheimer's disease. In this study selected components of essential oils, which carry a variety of important functional groups, were tested for their in-vitro anti-acetylcholinesterase activity.
Methods In-vitro anti-acetylcholinesterase activity was measured by an adapted version of Ellman's colorimetric assay.
Key findings 1,8-cineole, carvacrol, myrtenal and verbenone apparently inhibited AChE; the highest inhibitory activity was observed for myrtenal (IC50 = 0.17 mM). This is the first study showing the AChE inhibitory activity of myrtenal.
Conclusions Our investigations provided evidence for the efficacy of monoterpenes as inhibitors of AChE.
Keywords acetylcholinesterase inhibitor; Alzheimer's disease; monoterpenes; myrtenal

Introduction

Alzheimer's disease (AD) is a neuro-degenerative disorder and the most common form of dementia in the elderly.[1] These progressive degenerative brain syndromes affect memory, thinking, behaviour and emotion. Common symptoms are loss of memory, difficulties in finding the right words or understanding what people are saying, as well as difficulties performing previously routine tasks and even personality and mood changes.[2] During the course of AD, nerve cells die in particular regions of the brain, especially in the cerebral cortex. The brain shrinks as gaps develop in the temporal lobe and hippocampus, areas that are responsible for storing and retrieving new information. This in turn affects people's ability to remember, speak, think and make decisions.[2]

Today, more than 35 million people suffer from AD worldwide and AD is one of the leading causes of death in the United States.[3] In 2050, 100 million patients will live with AD worldwide.[4] These facts suggest that potent drugs are needed to slow down the progression of AD or even prevent it.

Several medications for treating AD are on hand, most of them inhibiting acetylcholinesterase (AChE). AChE cleaves acetylcholine (ACh) in the synaptic cleft. ACh is a neurotransmitter that is crucial for the signal transduction in the central nervous system. In AD patients the level of ACh is greatly reduced.[5] Through the inhibition of AChE, more acetylcholine is available, resulting in an improvement of cognitive function.[6] The therapy with AChE inhibitors (AChEi) is no longer considered to be only symptomatic, but also disease modifying,[7,8] and these medications are still the first choice for treating AD patients in the early stages of the disease.

One of the AChE compounds in use is galantamine (Reminyl®), an alkaloid isolated from various members of Amaryllidaceae (*Galanthus* spp., *Leucojum* spp. and *Narcissus* spp.) that has pronounced anti-cholinesterase activity.[9] It can therefore be assumed that plants might be a promising source of new bioactive compounds with anti-AChE activity.

The potential of natural products as inhibitors of AChE has already been identified and is under research.[10–12] We were able to show that four of the 34 essential oil components derived from various plants inhibited AChE *in vitro*. The active monoterpenoids provide lead structures that could be used to design optimised analogues with increased potency.

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Furthermore, this is the first report elucidating the potential of myrtenal (present in the essential oil of *Artemisia annua* L. (Asteraceae)[13] or *Glycyrrhiza glabra* L. (Fabaceae)[14]) as an inhibitor of AChE.

Material and Methods

Chemicals

1,8-cineole was purchased from Carl Roth Chemical Company (Karlsruhe, Germany). E-Anethole, allyl anisole, m-anisaldehyde, (−)-borneol, (−)-camphene, (+)-camphene, (+)-camphor, carvacrol, (−)-carvone, E-cinnamaldehyde, Z-methyl-cinnamate, cinnamyl alcohol, E/Z-citral (+/−)-citronellal, (+/−)-β-citronellol, eugenol, E/Z-iso-eugenol, methyl-eugenol, geraniol, (+/−)-limonene, (+/−)-linalool, (−)-menthone, β-myrcene, (+/−)-myrtenal, nerol, Z-nerolidol, 2-phenylethanol, (+/−)-α-pinene, (+/−)-β-pinene, (−)-terpinen-4-ol, (−)-α-thujone, thymol and (1S)-(−)-verbenone were bought from Fluka/Sigma-Aldrich (Steinheim, Germany).

AChE assay

To assess AChE inhibition, an adapted version of the Ellmann assay[15] in 96-well plates was used. A master mix consisting of 25 µl acetylthiocholine iodide (15 mM in phosphate buffer pH 7, Sigma-Aldrich), 125 µl dithionitrobenzoic acid (3 mM in phosphate buffer pH 8, Sigma-Aldrich) and 50 µl phosphate buffer (50 mM, pH 8) was prepared and added to 5 µl of essential oil components per well. Two known AChEis, physostigmine and galantamine (5 mg/100 µl in phosphate buffer pH 8, Sigma-Aldrich), served as positive controls, the solvent DMSO (J. T. Baker, Griesheim, Germany) as negative control.

After shaking for 20 s, measurements at times t = 0, 3, 6, and 9 min were recorded at 450 nm using the BioTek EL808 plate reader to avoid any interference in the results from spontaneous activity. After the 9 min reading, 25 µl of AChE (from electric eels, 3 U/ml in phosphate buffer pH 8, Sigma-Aldrich) was added to each well and the plate was left to incubate at room temperature for 3 min. After shaking for 20 s a final reading was recorded at 450 nm. The inhibitory activity was calculated in comparison to the negative control. Potential effects were expressed as a percentage of inhibition.

Statistical analysis

All experiments were carried out in triplicates and repeated at three individual days. All data are expressed as mean ± standard error ($n = 3$). The IC50 values were calculated using a four-parameter logistic curve (SigmaPlot® 11.0) representing 50% reduction of activity. Statistical analysis of the effects of increasing concentrations of essential oil components on the activity of AChE was performed using the using the non-parametric Kruskal–Wallis ANOVA test to compare the inhibitory activities of the four active monoterpenes. A significance level of $P \leq 0.05$ denoted significance in all cases. A post-hoc comparison of the individual differences between the various monoterpenes and phenylpropanoids was performed using the Dunn's test. Significance was detected in all cases except for the combination of carvacrole and myrtenal.

Results and Discussion

Secondary plant metabolites represent a wide variety of chemical compounds that interfere with different targets of the cell. Among these, more than 2500 monoterpenes and 2000 phenylpropanoids are known.[16] Monoterpenes exhibit cytotoxic,[17] anti-cancer,[18] antimicrobial,[19] anti-inflammatory, anti-viral[20] and antinociceptive,[21] sedative, relaxant,[22] as well as neuroprotective[23] effects.

In our study 34 essential oil components (monoterpenes, phenylpropanoids) were chosen as characteristic examples of various functional groups (methylene, hydroxyl, aldehyde groups, etc.). All compounds were tested for their in-vitro anti-acetylcholinesterase activity. Physostigmine and galantamine, both known acetylcholinesterase inhibitors,[24] were used as the positive controls (IC50 = 8.14 ± 0.98 and 15.07 ± 0.74 µM). Samples were considered to be inactive (NA) in the AChE assay if they showed less than 80% of AChE inhibition at a concentration of 25 mg/ml. Four essential oil components showed substantial inhibition of AChE activity: carvacrol, 1,8-cineole, myrtenal and verbenone (Table 1). The inhibitory potential of these compounds was compared to the already known AChE inhibitors physostigmine and galantamine. Post-hoc statistical analysis revealed that no significant difference between carvacrol and myrtenal was observed, and therefore these two chemical compounds should be considered to be equally active. Figure 1 shows the AChE inhibitory potential, which decreases in the following order: galantamine ≥ myrtenal ≥ carvacrol ≥ 1,8-cineole ≥ verbenone.

Myrtenal (Figure 2c) exhibited a comparatively strong AChE inhibition potential, with IC50 = 0.17 ± 0.01 mM, and might therefore be considered to be an interesting lead compound. Its comparatively strong inhibitory potential may be attributed to its aldehyde group, which may bind to free amino or sylfhydryl groups of AChE, forming a covalent modification through Schiff's base formation.[12] This is the first report elucidating the AChE inhibitory activity of myrtenal.

Carvacrol (Figure 2b) is a monoterpene of the essential oil of *Origanum vulgare* L. (Lamiaceae) and *Thymus vulgaris* L. (Lamiaceae). Its inhibitory effect on AChE activity has been shown previously.[25,26] The interaction of carvacrol with AChE is most likely based on the phenolic hydroxyl group, which binds to proteins, leading to a conformational change and therefore a loss of function.

1,8-cineole or eucalyptol (Figure 2a) is a secondary metabolite found in the essential oil of *Eucalyptus globulus* Labill (Myrtaceae) and other *Eucalyptus* species, different *Artemisia* species, as well as in *Cinnamomum cassia* Nees (Lauraceae). The monoterpenes camphor and 1,8-cineole are known AChE inhibitors[27,28] and a number of pure monoterpenes have been tested for AChE activity. It was found that 1,8-cineole and carvacrol have AChE-inhibitory activity and the oil extracted from a whole plant produced a more potent inhibition of AChE than the pure compounds alone.[25] Verbenone (Figure 2d) is less active, with IC50 = 2.66 ± 1.04 mM. This may be attributed to the fact that the presence of oxygenated functional groups decreases the AChE-inhibitory activity.[12]

Table 1 AChE-inhibitory activity of essential oil components. Samples were considered to be inactive (NA) in the AChE assay if they showed less than 80% inhibition of AChE activity at a concentration of 25 mg/ml. All data are expressed as mean ± SD of three individual experiments, each carried out in triplicate. Significance: *indicates a P value <0.05.[x]Post-hoc analysis detected no significant difference between AChE inhibition of carvacrol and (+/−)-myrtenal

Sample	AChEi (IC50 [mM])	Chemical class/functional group
E-Anethole	NA	Phenylpropanoid/methoxy group
Allyl anisole	NA	Phenylpropanoid/terminal methylene group
m-Anisaldehyde	NA	Phenylpropanoid/aldehyde
(−)-Borneol	NA	Monoterpene/alcohol
(−)-Camphene	NA	Monoterpene/exocyclic methylene group
(+)-Camphene	NA	Monoterpene/alkene
(+)-Camphor	NA	Monoterpene/ketone
Carvacrol	0.21 ± 0.04*, x	Monoterpene/phenolic OH-group
(−)-Carvone	NA	Monoterpene/ketone
1,8-Cineole	0.84 ± 0.19*	Bicyclic monoterpene
E-Cinnamaldehyde	NA	Phenylpropanoid/aldehyde
Z-Methylcinnamate	NA	Phenylpropanoid/ester
Cinnamyl alcohol	NA	Phenylpropanoid/alcohol
E/Z-Citral	NA	Monoterpene/aldehyde
(+/−)-Citronellal	NA	Monoterpene/aldehyde
(+/−)-β-Citronellol	NA	Monoterpene/alcohol
Eugenol	NA	Phenylpropanoid/phenolic, terminal methylene group
E/Z-Iso-eugenol	NA	Phenylpropanoid/phenolic
Methyleugenol	NA	Phenylpropanoid/terminal methylene group
Geraniol	NA	Monoterpene/alcohol
(+/−)-Limonene	NA	Monoterpene/alkene
(+/−)-Linalool	NA	Monoterpene/alcohol
(−)-Menthone	NA	Monoterpene/ketone
β-Myrcene	NA	Monoterpene/methylene groups
(+/−)-Myrtenal	0.17 ± 0.01*, x	Monoterpene/aldehyde
Nerol	NA	Monoterpene/alcohol
Z-Nerolidol	NA	Sesquiterpene/alcohol
2-Phenylethanol	NA	Pneylpropanoid/alcohol
(+/−)-α-Pinene	NA	Monoterpene
(+/−)-β-Pinene	NA	Monoterpene
(−)-Terpinen-4-ol	NA	Monoterpene/alcohol
(−)-α-Thujone	NA	Monoterpene/ketone, cyclopropane ring
Thymol	NA	Monoterpene/phenolic OH-group
(1*S*)-(−)-Verbenone	2.66 ± 1.04*	Monoterpene/ketone

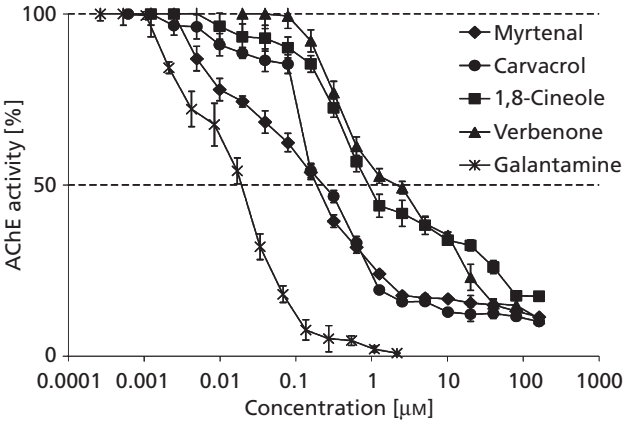

Figure 1 Dose–response curve for acetylcholinesterase inhibitors from essential oil components. Positive control: galantamine. All data are expressed as mean ± SD of three individual experiments, each carried out in triplicate.

The LD50 toxicity values (oral, rat) for these compounds are[29] myrtenal 14.91 mM/kg, carvacrol 5.25 mM/kg, 1,8-cineole 16.08 mM/kg, verbenone 5.51 mM/kg. Considering that these toxicity values are much higher (by factors ranging from 2 to 85) than the IC50 values of AChE inhibition, *in vivo* studies should follow to elucidate the potential of these compounds as inhibitors of AChE.

Conclusions

Natural products are an interesting source of inhibitors of AChE. We were able to show that four of the 34 essential oil components derived from various plants inhibited AChE *in vitro*: 1,8-cineole, carvacrol, myrtenal and verbenone. Furthermore, this is the first report elucidating the potential of myrtenal (present in the essential oil of *Artemisia annua* L. (Asteraceae)[13] or *Glycyrrhiza glabra* L. (Fabaceae))[14] as an inhibitor of AChE.

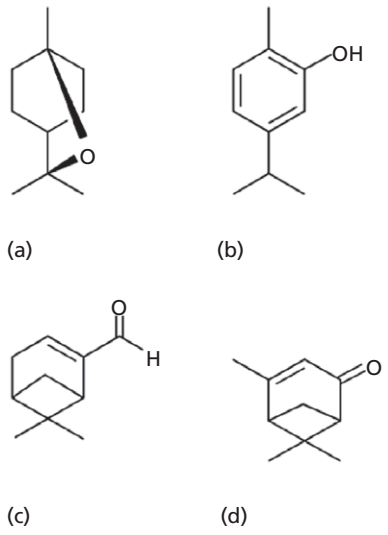

Figure 2 Chemical structures of 1,8-cineole (a), carvacrol (b), myrtenal (c) and verbenone (d).

Declarations

Conflict of interest

The Authors declare that they have no conflicts of interest to disclose.

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